EXPRESSION OF DSRAB IN DESULFOTALEA PSYCHROPHILA SUBJECTED TO SIMULATED MARTIAN CONDITIONS.

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BACKGROUND

The ability of microorganisms to survive and proliferate under extreme conditions such as those present in today's Mars is still unknown. Furthermore, recent discoveries of the Martian soil composition indicate the existence of areas with high concentrations of different sulfate compounds such as calcium sulfate ($CaSO_4$), magnesium sulfate ($MgSO_4$), and iron sulfates ($Fe(SO_4)_2$, $Fe_2(SO_4)_3$)[1,2, 3].

In addition, the increasing interest for Astrobiological studies demands the availability of reliable techniques that allow us to recognize bacterial activity. Therefore, simulated experiments along with molecular markers offer the possibility to recreate and understand biological phenomena associated to active bacterial metabolism[8].

In this research, a combination of cultural and molecular biology techniques has been used to identify metabolic activity of a psychrophilic sulfate-reducing bacterium named *Desulfotalea psychrophila (D. psychrophila)*[9]. Furthermore, different types and concentrations of sulfate compounds like those present in the Martian soil have been used to target a molecular marker present in this microbe. The latter, also known as the dsrAB operon, encodes the genes required for the biosynthesis of the dissimilatory sulfite reductase (DSRAB enzyme) which intervenes in the production of cellular energy that occurs at the last step of sulfate reduction [4,5,6,10].

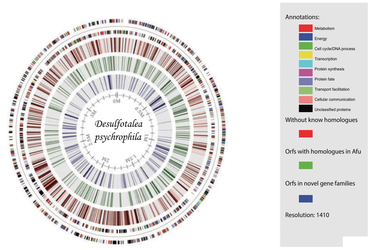

Figure 1. *D. psychrophila* chromosome showing functional genes/proteins. Illustration extracted from Rabus et al., 2004.

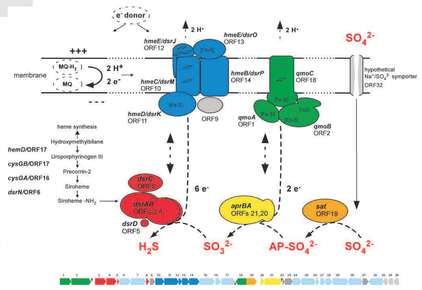

Figure 2. Sulfate reduction process in bacterial cells. In red: Dissimilatory sulfite reductase (DSRAB) which transfers six electrons from a donor to sulfite generating hydrogen sulfide (H_2S) and energy (ATP). Illustration extracted from Mussmann et al., 2005.

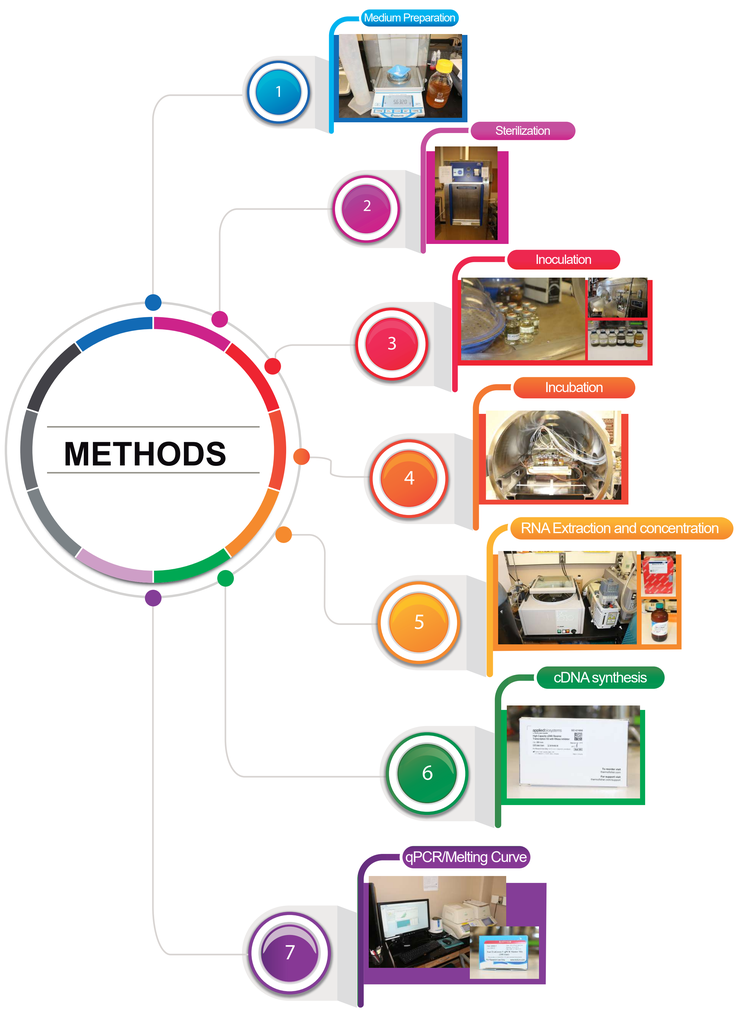

Table 1. *D. psychrophila* culture conditions and sulfate compounds (Replacement of terminal electron acceptor on DSMZ141 optimal medium). Final medium volume of 20 mL, and anaerobic conditions (mixture of H_2 and CO_2 as stated on DSMZ141).

Nº	Sulfate compound	Concentration	Incubation Temperature
1	$MgSO_4$ (Positive control)	0.345 wt %	10º C and 0º C
2	$CaSO_4$	0.1 wt %	10º C and 0º C
3	$MgSO_4$	10 wt %	10º C and 0º C
4	$MgSO_4$	18 wt %	10º C and 0º C
5	$Fe(SO_4)_2$	10 wt %	10º C and 0º C
6	$Fe(SO_4)_2$	14 wt %	10º C and 0º C
7	$Fe_2(SO_4)_3$	10 wt %	10º C and 0º C
8	$Fe_2(SO_4)_3$	20 wt %	10º C and 0º C
9	$Fe_2(SO_4)_3$	30 wt %	10º C and 0º C
10	$Fe_2(SO_4)_3$	40 wt %	10º C and 0º C
11	$Fe_2(SO_4)_3$	48 wt %	10º C and 0º C

RESULTS

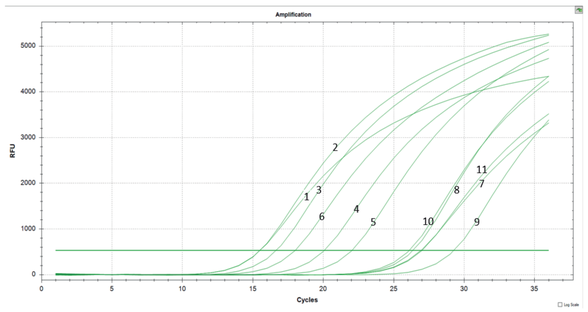

Figure 3. Fluorescence detection of dsrAB operon (active metabolism) from cultures of *D. psychrophila* grown in different types and concentrations of sulfate compounds. Incubation at 10ºC. **1**:$MgSO_4$ 0.345 wt %; **2**:$CaSO_4$ 0.1 wt %; **3**:$MgSO_4$ 10 wt %; **4**:$MgSO_4$ 18 wt %; **5**:$FeSO_4$ 10 wt %; **6**:$FeSO_4$ 14 wt %; **7**:$Fe_2(SO_4)_3$ 10 wt %; **8**:Fe2$(SO_4)_3$ 20 wt %; **9**:$Fe_2(SO_4)_3$ 30 wt %; **10**:$Fe_2(SO_4)_3$ 40 wt %; **11**:$Fe_2(SO_4)_3$ 48 wt %.

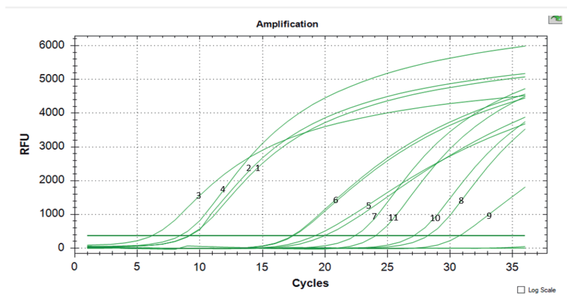

Figure 4. Fluorescence detection of dsrAB operon (active metabolism) from cultures of *D. psychrophila* grown in different types and concentrations of sulfate compounds. Incubation at 0ºC. **1**:$MgSO_4$ 0.345 wt %; **2**:$CaSO_4$ 0.1 wt %; **3**:$MgSO_4$ 10 wt %; **4**:$MgSO_4$ 18 wt %; **5**:$FeSO_4$ 10 wt %; **6**:$FeSO_4$ 14 wt %; **7**:$Fe_2(SO_4)_3$ 10 wt %; **8**:Fe2$(SO_4)_3$ 20 wt %; **9**:$Fe_2(SO_4)_3$ 30 wt %; **10**:$Fe_2(SO_4)_3$ 40 wt %; **11**:$Fe_2(SO_4)_3$ 48 wt %.

Table 2. Quantification cycle values (Cq) for samples of *D. psychrophila* grown in different types and concentrations of sulfate compounds.

Nº	Sulfate compound	Concentration	10º C	0 ºC
1	$MgSO_4$ (Positive control)	0.345 wt %	15.49	9.55
2	$CaSO_4$	0.1 wt %	15.48	9.36
3	$MgSO_4$	10 wt %	16.63	6.40
4	$MgSO_4$	18 wt %	20.07	9.10
5	$Fe(SO_4)_2$	10 wt %	22.04	19.58
6	$Fe(SO_4)_2$	14 wt %	18.01	17.41
7	$Fe_2(SO_4)_3$	10 wt %	27.01	22.67
8	$Fe_2(SO_4)_3$	20 wt %	26.34	28.88
9	$Fe_2(SO_4)_3$	30 wt %	29.25	30.92
10	$Fe_2(SO_4)_3$	40 wt %	26.09	26.78
11	$Fe_2(SO_4)_3$	48 wt %	26.93	24.24

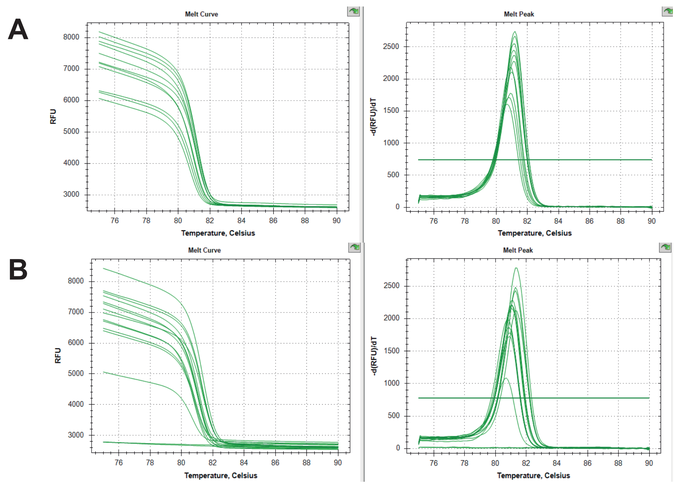

Figure 5. Melting curve points of all samples involved in experiments at 10º C **(A)** and 0 ºC **(B)**.

CONCLUSIONS

- Our experimental design is suitable for detection of bacterial active metabolism in cultures subjected to different types and concentrations of sulfate compounds and temperatures (See Melting points in Figure 5). Further studies are required to determine if the same platform is suitable for experiments at low pressure.

- It is particularly interesting that *D. psychrophila* metabolic activity is higher at lower than optimal incubation temperatures (See Cq values in Table 2, Figures 3 and 4).

- *D. psychrophila* can metabolize at most conditions used in this study. Furthermore, lower sulfate concentrations favor higher metabolic rates ($MgSO_4$ at 10º C). However, as it is shown in Table 2 for $MgSO_4$ at low temperatures, higher concentrations favor higher metabolic rates (See Cq values in Table 2, Figures 3 and 4).

- In general, $CaSO_4$ seems to slightly increase bacterial proliferation in comparison to the positive control regardless of temperature (Table 2). Furthermore, dsrAB expression in cultures with $Fe(SO_4)_2$ seem to be higher at increasing concentrations (See Table 2, Figure 3 and 4).

- In general, there is not much difference in samples grown with $Fe_2(SO_4)_3$ at any of the temperatures used in the study (Table 2, Figure 3 and 4).

ACKNOWLEDGMENTS

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